Studies on Larval Feeding Stimulant of Zizeeria maha (Kollar) (Lepidoptera: Lycaenidae) in Oxalis corniculata L.: Elucidation of Triadic Relationships among Light Environment, Host Plant Quality, and Feeding Behavior

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Summary

Studies on Larval Feeding Stimulant of *Zizeeria maha* (Kollar)
(Lepidoptera: Lycaenidae) in *Oxalis corniculata* L.:
Elucidation of Triadic Relationships among Light Environment,
Host Plant Quality, and Feeding Behavior

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Almost all the plant species must adapt to the environment in which they grow, because they cannot migrate easily to other places except for the seed dispersal. Based on this fact, one can readily imagine that physicochemical differences can occur among individuals or patches, even in the same plant species, with differences in environmental properties. Similarly, it is difficult for a phytophagous larva dependent on a specific host plant to move actively from its hatching site to a more nutritious plant for its continued growth. We may thus expect a triadic interaction among environment, plant quality, and behavior or preference of the herbivore. However, no case study has demonstrated these interactions concretely. The object of the present study was to evaluate the triadic interaction experimentally, first by identifying feeding stimulants of *Zizeeria maha* (Lepidoptera: Lycaenidae) larva in its host plant *Oxalis corniculata* L. (*Oxalis*: Oxalidaceae) and then by relating the light environment of *O. corniculata* to the physicochemical properties of *O. corniculata*, especially with respect to feeding stimulant(s), and to the larval feeding behavior of *Z. maha*.

First, we identified a feeding stimulant of *Z. maha* larva in its host plant *O. corniculata* using an artificial diet. The control artificial diet did not promote larval feeding. Larvae were strongly stimulated to feed on artificial diets containing a crude methanol extract of host plant leaves. Fractionation and bioassays revealed that the strongest feeding stimulating activity was retained in the water layer, in which oxalic acid was the major compound detected. Oxalic acid accounted for as much as 15 wt% of the methanolic extract and more than 90% of extracted oxalic acid was found in the water layer. Removal of oxalic acid as calcium oxalate precipitate from the water layer resulted in a significant decrease of feeding activity in the filtrate. Re-addition of standard oxalic acid to the inactive filtrate restored feeding activity. Addition of oxalic acid in the range of 3.15 – 6.30 mmol, corresponding to 0.5 – 1.0 g of fresh leaves of *Oxalis*, to 1 g of artificial diet significantly stimulated feeding compared to the intact artificial diet. Oxalic acid was concluded to be the major feeding stimulant for *Z. maha* larvae.

Second, the relationship between light environment and the physicochemical properties of *O. corniculata* was investigated in the field.

O. corniculata leaves were collected from seven study areas with different light intensities, and inorganic elements, organic acids, and phenolics were analyzed. The major organic acid and phenolic compound were determined to be oxalic acid and apigenin glycosides, respectively. These

compounds showed significant differences among study areas and positive correlations of concentration with light intensity. In contrast, water content and inorganic elements showed no differences among the study areas and no correlation with light intensity.

Feeding responses of *Z. maha* larvae on leaves of *O. corniculata* collected from the seven study areas were tested. Of eight feeding parameters, four, namely frass weight, ingested amount, relative growth rate, and relative food consumption rate showed positive correlations with oxalic acid content in leaves. From the relationships between light intensity and oxalic acid content in the leaves, a triadic relationship among light intensity, oxalic acid, and larval feeding response was hypothesized: higher light intensity leads to higher content of oxalic acid in leaves, resulting in higher feeding response.

Next, the above hypothesis was tested under laboratory conditions, using *O. corniculata* cultivated in two different light environments. Chronological sampling showed that oxalic acid content declined in leaves cultivated under the dark condition. After 4 weeks, oxalic acid content in leaves cultivated under the light condition was approximately twice (L1: 2.61 μg, L2: 2.38 μg) that under the dark condition (D1: 0.92 μg, D 2: 0.84 μg). Thus, it was confirmed that light intensity influenced the quantity of the major secondary metabolite, oxalic acid, in *O. corniculata*. Likewise, larval feeding activity was higher when larvae were fed with leaves grown under the light condition, containing higher amounts of oxalic acid. This result showed that oxalic acid content in *O. corniculata* was positively correlated with light intensity and with larval feeding activity of *Z. maha* under laboratory conditions. This is the first demonstration in *O. corniculata* that the production of secondary metabolites such as oxalic acid correlates positively with light intensity in both field and laboratory.

From these results, it was concluded that the triadic relationship among light environment, *O. corniculata* and *Z. maha* could be described as follows. Increased light intensity induces *O. corniculata* to raise the production of oxalic acid as a defensive secondary metabolite in the leaves. Larvae of *Z. maha*, a specialist herbivore of *O. corniculata*, prefers to feed on leaves grown in a light environment because they are stimulated by the oxalic acid content in the leaves. We propose a triadic relationship based on the identification of oxalic acid in *O. corniculata* as a feeding stimulant for *Z. maha* larvae, evaluation of the phytochemical effect of light intensity on *O. corniculata*, and the positive correlation between light intensity during host plant growth and larval feeding activity.